

中国秃疮花属和海罂粟属的细胞学研究*

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摘要: 报道了秃疮花属 3 种, 即莖叶秃疮花、秃疮花和宽果秃疮花共 11 个居群及其姐妹类群海罂粟属 1 种 (海罂粟) 一个居群的染色体数目。研究结果表明, 秃疮花属全部三种的染色体数目均为 $2n=12$; 海罂粟的染色体数目为 $2n=10$, 并且是该属中新发现的最小数目。

关键词: 秃疮花属; 海罂粟属; 罂粟科; 染色体数目

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Cytology of *Dicranostigma* and *Glaucium* (Papaveraceae) from ChinaXIANG Chun-lei¹, DONG Hong-jin², HU Guo-xiong¹, Nadia B. Zahra³, Tsuneo FUNAMOTO^{4**}

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Abstract: The cytological studies were performed in 11 populations of *Dicranostigma lactucoides*, *D. leptopodum*, and *D. erectum* and one population for *Glaucium fimbriigerum*. All three species of *Dicranostigma* possessed chromosome number $2n=2x=12$, of which chromosome numbers for *D. erectum* and *D. lactucoides* are reported here for the first time. *Glaucium fimbriigerum* presented the number $2n=2x=10$, which is not only new to the genus but also the known lowest number for *Glaucium*.

Key words: *Dicranostigma*; *Glaucium*; Papaveraceae; Chromosome number

There are approximately 40 genera and 800 species in family Papaveraceae (Judd *et al.*, 1999). This family has gained special attention for its pharmaceutical, ornamental, and alimentary importance due to the presence of high content of alkaloids and a number of species which are of horticultural interest (Wu and Chuang, 1999). There are 440 species of Papaveraceae belonging to 19 genera presently reported from China. This large occurrence has made

China as an important distribution center of this family (Wu and Chuang, 1999; Zhang *et al.*, 2008).

Chromosome numbers in Papaveraceae vary greatly along with basic numbers, and with cytological variability in different members of the family. However, basic chromosome numbers are usually the multiples of $x=6, 7, 8, 9, 10$ and 11 and rarely of other numbers (Kadereit, 1993). Chromosome numbers for some Chinese genera are reported by differ-

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ent studies (Krahulcova, 1982; Li and Hsu, 1986; Huang *et al.*, 1989; Safonova, 1989, 1991, 1994; Zhang and Fu, 1993; Xu and Wei, 1994; Meng *et al.*, 2006). However, the relevant cytological information for *Dicranostigma* J. D. Hooker & Thomson and its sister group *Glaucium* Miller is very scarce.

Dicranostigma is a small genus in subfamily Chelidonioideae. As currently circumscribed (Zhang *et al.*, 2008), the genus consists of three species, of which *D. lactuoides* Hook. f. & Thomson is found in Himalayas and *D. erectum* K.-F. Günther (= *D. platycarpum* C. Y. Wu; Dong and Xiang, 2015) and *D. leptopodum* (Maxim.) Fedde are present in the Hengduan Mountains and Losses Plateau region. Safonova (1991) and Wu and Wang (1997) reported chromosome number $2n=12$ for *D. leptopodum* and *D. henanense* S. Y. Wang & L. H. Wu, respectively. However, the latter was suggested to be a synonym of the former species (Zhang *et al.*, 2008). Therefore, chromosome data is only available for *D. leptopodum* to date, and the variability for chromosome number within the genus is still unknown.

Glaucium Miller has been shown as sister to *Dicranostigma* (Brückner, 1992; Hoot *et al.*, 1997). The genus consists of 23 species, which occur around the Mediterranean. In China, only three species were recorded from Xinjiang (Chuang, 1993; Wu and Chuang, 1999). The chromosome numbers for seven species belonging to the genus *Glaucium* have already been reported by different authors (Amin, 1979; Mory, 1979; Ceschmedziev, 1983; Ghaffari and Chariat-Panahi, 1985; Ghaffari and Kelich, 2006;

Ruiz de Clavijo, 1990; Safonova, 1991, 1993, 1994; Runemark, 2000). These are *G. corniculatum* (L.) Rudolph, *G. elegans* Fisch. & C. A. Mey., *G. flavum* Crantz, *G. fimbriigerum* Boiss., *G. leiocarpum* Boiss., *G. oxylobum* Boiss. & Buhse, and *G. squamigerum* Kar. & Kir. Unexceptionally, the chromosome number for all species is $2n=12$ (Table 1). The chromosome numbers seemed to be constant in *Glaucium*, but there is not enough cytological data reported in *Dicranostigma*. Thus further cytological studies as well as morphological and molecular data are needed.

As part of our studies for taxonomical, systematics and population genetics of the genus *Dicranostigma* and related genera, we investigated the cytological study of 11 populations of three *Dicranostigma* species and one population of *Glaucium fimbriigerum* from China and the results are presented here. The aims of the present study were to determine chromosome numbers in *Dicranostigma* and *Glaucium* in China, and to test the variability of chromosome numbers within different populations of these genera. This will contribute our knowledge for future systematic study of this group.

1 Materials and method

The seeds of *Dicranostigma* and *Glaucium fimbriigerum* for the chromosome study were collected from field in 2014 (Table 2), and voucher specimens are kept at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Seeds were sown on wet filter paper in Petri dishes for germination and incubated at 23–25 °C in Temperature

Table 1 List of taxa, location and chromosome number reported previously

No.	Taxon	Chromosome number ($2n$)	References
1	<i>Dicranostigma leptopodum</i>	12	Safonova (1991, 1994)
2	<i>Glaucium corniculatum</i>	12	Amin (1979); Ruiz de Clavijo (1990)
3	<i>G. elegans</i>	12	Mory (1979); Ghaffari and Chariat-Panahi (1985)
4	<i>G. flavum</i>	12	Baltisberger and Voelger (2006); Runemark (2000)
5	<i>G. fimbriigerum</i>	12	Safonova (1991); Ghaffari and Kelich (2006)
6	<i>G. leiocarpum</i>	12	Ceschmedziev (1983)
7	<i>G. oxylobum</i>	12	Safonova (1991)
8	<i>G. squamigerum</i>	12	Safonova (1991)

Table 2 List of taxa, location and chromosome number examined in this study

No.	Taxon	Chromosome number ($2n$)	Collection data
A	<i>Glaucium fimbrilligerum</i>	10	Tongde, Qinghai; Xiang Chunlei <i>et al.</i> 1067 (KUN)
B	<i>Dicranostigma leptopodum</i>	12	Rangtang, Sichuan; Xiang Chunlei <i>et al.</i> 1045 (KUN)
C	<i>D. leptopodum</i>	12	Dangchang, Gansu; Xiang Chunlei <i>et al.</i> 1127 (KUN)
D	<i>D. leptopodum</i>	12	Tianshui, Gansu; Xiang Chunlei <i>et al.</i> 1120 (KUN)
E	<i>D. leptopodum</i>	12	Maerkang, Sichuan; Xiang Chunlei <i>et al.</i> 1042 (KUN)
F	<i>D. leptopodum</i>	12	Kangding, Sichuan; Xiang Chunlei <i>et al.</i> 1012 (KUN)
G	<i>D. leptopodum</i>	12	Maodian, Sichuan; Xiang Chunlei <i>et al.</i> 1159 (KUN)
H	<i>D. leptopodum</i>	12	Xiaojin, Sichuan; Xiang Chunlei <i>et al.</i> 1036 (KUN)
I	<i>D. leptopodum</i>	12	Shangri-La, Yunnan; Xiang Chunlei <i>et al.</i> 969 (KUN)
J	<i>D. leptopodum</i>	12	Lijiang, Yunnan; Xiang Chunlei <i>et al.</i> 928 (KUN)
K	<i>D. erectum</i>	12	Lijiang, Yunnan; Xiang Chunlei <i>et al.</i> 959 (KUN)
L	<i>D. luctuoides</i>	12	Nielamu, Xizang; Dong Hongjin and Hu Guoxiong 992 (KUN)

Gradation Chamber (TG100AD, Nippon Medical & Chemical Instruments Co., LTD). Chromosome observations were made in meristematic cells of freshly growing root tip cells. Freshly growing root tips were obtained from germinated plants. They were pretreated in $2 \text{ mmol} \cdot \text{L}^{-1}$ 8-hydroxyquinoline for 4 hrs at *ca.* 20 °C and then fixed 45% acetic acid for 10 min at *ca.* 2 °C. Finally they were macerated in a mixture of 45% acetic acid and $1 \text{ mol} \cdot \text{L}^{-1}$ hydrochloric acid (1:1) for 17–18 s at *ca.* 60 °C. The macerated seeds were stained in 2% aceto-orcin for over 30 min at room temperature in a moist chamber with 45% acetic acid (see Funamoto and Ogawa, 2014; Xiang *et al.*, 2014).

Slides were prepared by the conventional squash method. The slides were faintly heated under an alcohol flame for 1–2 s before observation. Photographs were taken using OLYMPUS Digital Camera (DP72SETA2) System. Classification of somatic metaphase chromosomes of centromeric position for *Glaucium* followed Levan *et al.* (1964): Arm ratio was calculated by long arm/short arm, M=median (1.0), m=median (1.0<m<1.7), sm=submedian (1.7≤sm<3.0), st=subterminal (3.0≤st<7.0), t=terminal (≥7.0).

2 Results and discussion

The chromosome number of *Glaucium fimbri-ligerum* from population of Tongde, Qinghai, China,

is $2n=2x=10$ (Fig. 1, A). This number is reported here for the first time for this genus. The length of *Glaucium fimbri-ligerum* chromosome ranged from 2.7 to 4.0 μm long, and haploid chromosome length was 31.8 μm long. The chromosome complement at mitotic metaphase consisted of 6 median region, 4 submedian region chromosomes. No satellite chromosomes were observed in this population.

Safonova (1993) first reported that chromosome number for *Glaucium fimbri-ligerum* is $2n=2x=12$. Recently, the parallel findings have been found by Ghaffari and Kelich (2006) in their cytological studies for Iranian angiosperm plants. Besides, all previous studies showed that chromosome numbers for *Glaucium* species are $2n=2x=12$ (Ceschmedziev, 1983; Ghaffari and Chariat-Panahi, 1985; Ruiz de Clavijo, 1990; Safonova, 1993; Runemark, 2000). Incorporating these findings with our result here, it is evident that two different base numbers occur in *Glaucium*: $x=5$ and 6, but all these previous results revealed that the base chromosome number $x=6$ is more frequent in *Glaucium*. It's also worth noting that the basic chromosome number $x=5$ is rare in Papaveraceae. To our knowledge, this number was only reported from *Chelidonium asiaticum* Krahulcova (Krahulcova, 1982; Shatalova, 2000), *C. majus* L. (Starodubtsev, 1989; Sha *et al.*, 1995; Yan *et al.*, 2000), *Macleaya cordata* (Willd.) R. Br. and *M. microcarpa* (Maxim.) Fedde (Safonova, 1989).

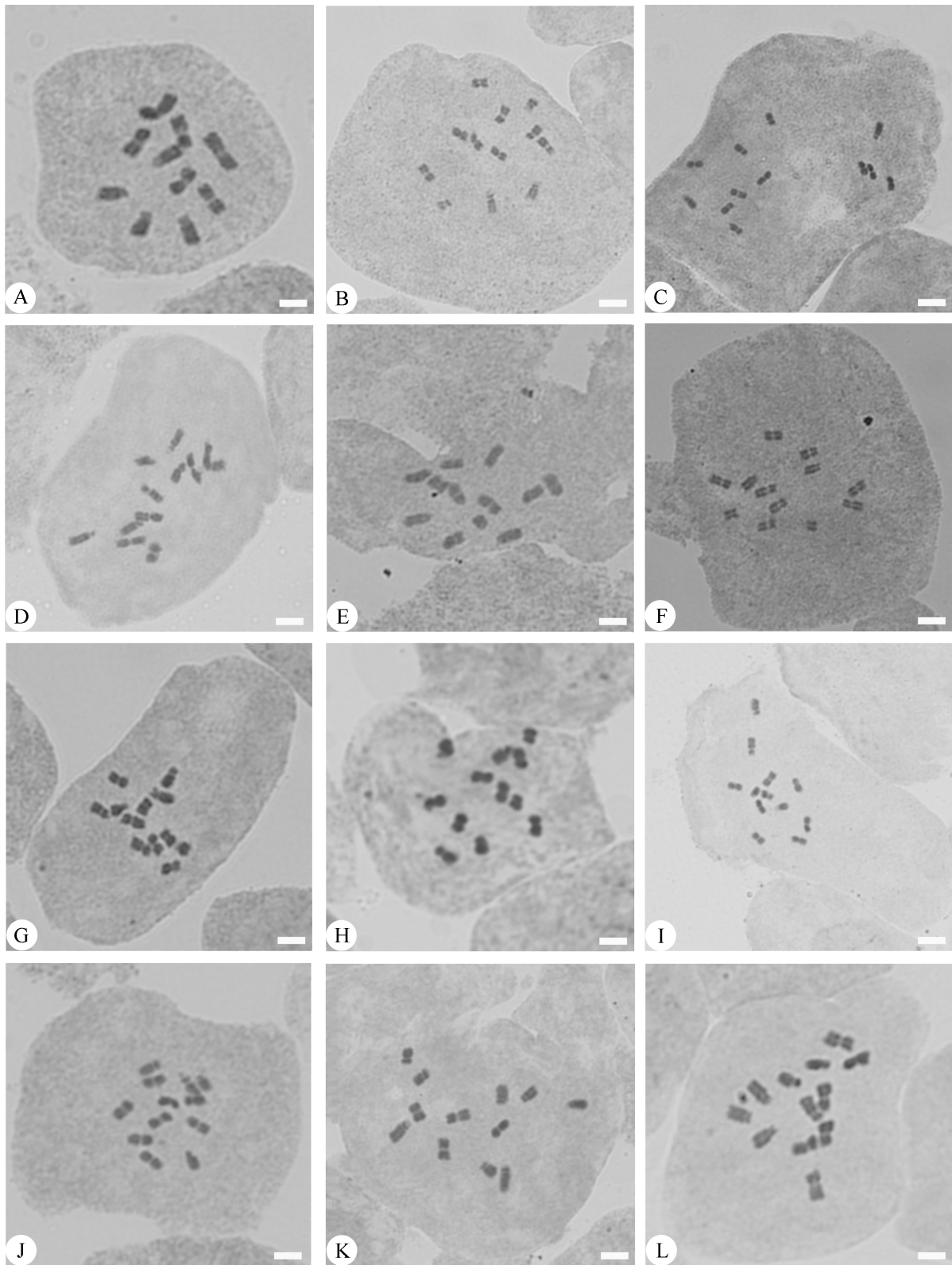


Fig. 1 Selected photomicrographs of somatic chromosomes at metaphase of *Dicranostigma* and *Glaucium* from China
 A. *Glaucium fimbriigerum* (Tongde, Qinghai); B-J. *Dicranostigma leptopodum* (B, Rangtang, Sichuan; C, Dangchang, Gansu;
 D, Tianshui, Gansu; E, Maerkang, Sichuan; F, Kangding, Sichuan; G, Maoxian, Sichuan; H, Xiaojin, Sichuan; I, Shangri-La,
 Yunnan; J, Lijiang, Yunnan); K. *D. erectum* (Lijiang, Yunnan); L. *D. luctuoides* (Nielamu, Xizang) (bar=5 μ m)

Most *Glaucium* species are distributed in Mediterranean area. In China *Glaucium* species are considered endemic to Xinjiang, here our collection of *Glaucium fimbrilligerum* from Qinghai constitutes the new record of the genus for Qinghai. In Xinjiang, these species grow in arid habitat, while in Tongde, Qinghai, the plants grow on high altitude and the habitat is cooler and wetter than in Xinjiang. The populations thus react to changes in their environment by adaptive modifications which may cause the chromosome number change. Here, the lower base number $x=5$ has possibly developed through descending dysploidy as an adaption to cool-wet habitat, although the process behind this phenomenon remains hypothetical in the absence of in-depth studies.

Chromosome number for all the three species of *Dicranostigma* is $2n=2x=12$ (Fig. 1, B–L) and chromosome numbers for *D. erectum* and *D. lactuoides* have never been reported earlier. The length of chromosome in all populations varied from 2 to 3 μm , which is too small to conduct a karyotype analysis.

For *Dicranostigma*, the chromosome numbers reported here corroborates with previous study (Safonova, 1991). In China, our three *Dicranostigma* species have a disjunct distribution and occupy different habitats. *Dicranostigma leptopodum* and *D. lactuoides* grow in dry-warm valley but *D. erectum* survives in high latitude and grows in alpine meadows and/or rock crevices. Considering polyploidization may play an important role in Papaveraceae distributed in the high altitude area, *D. erectum* is supposed to be a polyploid species. However, there are no differences in chromosome numbers among the *Dicranostigma* populations and all of them are diploid species. According to some investigations, polyploidization may play an important role in *Meconopsis* Vig. (Xie *et al.*, 2014) and *Corydalis* DC. in Papaveraceae and some other plants (Hong and Zhang, 1990; Yang, 2002; Zhou *et al.*, 2012). However, polyploidy is not common in the alpine flora of the Hengduan Mountains Hotspot, and the frequency of polyploidy is recorded to be only 22% in the area

(Nie *et al.*, 2005). Here, our study shows that polyploidization has not played an important role in the evolutionary history of the genus *Dicranostigma*.

Evidences from molecular data showed that *Dicranostigma* is sister to *Glaucium* (Gleissberg and Kadereit, 1999; Ruiz-Sanchez *et al.*, 2012). Additionally, morphological similarities between them such as growth form, sepals, leaf and fruit shape and seed morphology also shows that these genera are closely related. The similar chromosome numbers were reported for both genera, i. e. $2n=12$ in the previous investigations (Table 1). Therefore, Vent and Mory (1973) even though these two genera are congeneric. Although, our findings do not provide further support for their close relationship because they have different chromosome numbers. Hence, in future there is a need to conduct cytological studies including increased populations of other *Glaucium* species.

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